

## LETTER TO THE EDITOR

Dear Sir,

### Kaposi's sarcoma herpes virus and Kaposi's sarcoma in the elderly populations of 3 Mediterranean islands

Classical Kaposi's sarcoma (KS), in contrast to KS that occurs in patients with AIDS, in Africa or with iatrogenic immunosuppression, is geographically clustered in the Mediterranean basin, in Eastern Europe and in migrants from these areas.<sup>1</sup> It appears as a low-grade, indolent vascular neoplasm affecting preferentially elderly males (male-to-female ratio 3:1), with the highest incidence rate in the 6th through 8th decades of life. Kaposi's sarcoma herpes virus [KSHV, also known as human herpesvirus type 8 (HHV-8)], a newly discovered member of the gamma herpesviruses family, appears to be the primary etiological agent in classical as well as other forms of KS.<sup>2</sup> Since the development of sensitive and specific methods to detect antibodies to KSHV antigens, seroepidemiological studies have found increased KSHV seroprevalence rates in individuals at high risk for sexually transmitted diseases and in the general populations of several African and Mediterranean countries.<sup>3,4</sup> In these countries, KSHV infection seems to begin in childhood and to increase with age, reaching peak seroprevalence rates in people older than 50 years. KSHV seroprevalence in the elderly and its relationship to KS remain undefined.

In our study, we investigated whether age- and sex-specific KSHV seroprevalence rates paralleled classical KS incidence rates among elderly people living in 3 islands located in the central Mediterranean sea, i.e., Malta, Sicily and Sardinia.

In all 3 populations, residual sera samples were obtained following the completion of routine chemistry analyses of consecutive, elderly (age 65 or older) hospital patients in Malta (100 males, 100 females) and from outpatients in Palermo, Sicily (93 males, 60 females) and Sassari, Sardinia (51 males, 53 females). The study was reviewed and approved by the Office of Human Subjects Research of the National Institutes of Health and by the Institutional Review Boards of the National Cancer Institute, the Research Triangle Institute and the University of Palermo.

Data on the incidence of classical KS per 100,000 person-years ( $10^5 \cdot \text{year}$ ) in Sicily and Sardinia were obtained from the published literature.<sup>5,6</sup> Comparable data in Malta were calculated using the 1990 census and KS cases reported to the histopathology database, the National Cancer Registry and a survey of local physicians.<sup>7</sup>

Aliquots of sera were frozen and sent to the SAIC-National Cancer Institute Viral Epidemiology Laboratory (Frederick, MD). As previously described,<sup>8</sup> samples of sera were tested for KSHV antibodies by EIA at a 1:20 dilution against the K8.1 recombinant protein and by immunofluorescence assay (IFA) at a 1:100 dilution against latent nuclear antigens using uninduced BCP-1 cells.<sup>9</sup>

Mean ages were compared by ANOVA with 2-sided *p* values. Primary KSHV analysis was based on an algorithm using K8.1

and IFA.<sup>8</sup> Specifically, sera samples with a K8.1 optical density (OD)  $\geq 1.5$  were scored positive, those with a K8.1 OD  $< 0.5$  were scored negative and those with K8.1 OD from 0.5 to 1.5 were scored by their IFA result. Seroprevalence was determined by island and, within each island, by sex and age group (65–74 years, 75–84 years,  $\geq 85$  years). Multivariate logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (95% CI) of KSHV seropositivity adjusted for island, sex and median age (76 years). Results with the IFA alone and with an alternative K8.1 cutoff are presented briefly.

The 457 subjects included 244 men and 213 women of similar age (76 vs. 77 years, *p* = 0.21). Mean ages were 79 years in Malta, 76 years in Sicily and 73 years in Sardinia (*p* < 0.0001). KSHV seroprevalence rates were 8.5% in Malta, 20.3% in Sicily and 25.0% in Sardinia with the K8.1-IFA algorithm. Using only the K8.1 result (OD 1.0 cutoff), estimated seroprevalence rates were 9.0% in Malta, 19.6% in Sicily and 27.9% in Sardinia. By IFA alone, estimated seroprevalence rates were 16.0% in Malta, 27.5% in Sicily and 20.2% in Sardinia.

Within each island, KSHV seroprevalence was similar by sex and differed little by age among these elderly subjects (Table I). By multivariate logistic regression, KSHV seroprevalence was 3.25-fold (95% CI = 1.89–5.57) higher in Sicily and 4.07-fold (95% CI = 2.25–7.34) higher in Sardinia than in Malta. With the adjustment for island, it did not vary significantly by sex or age (Table II).

Incidence rates of classical KS among men (Table III) were higher in Sicily (6.2 per  $10^5 \cdot \text{year}$ ) and especially in Sardinia (8.8 per  $10^5 \cdot \text{year}$ ) compared with Malta (2.2 per  $10^5 \cdot \text{year}$  [upper 95% CI = 4.8 per  $10^5 \cdot \text{year}$ ]). In contrast, there was no difference by island in the incidence rates among women (1.8–2.5 per  $10^5 \cdot \text{year}$ ). If KS is assumed to develop only in KSHV-seropositive persons, the seropositive population at risk can be estimated from Table I. With these denominators for KSHV-seropositive elderly men, there was no difference in the annual rates of classical KS by island (each about 1:3,300 per year, Table III). Among KSHV-seropositive women, however, annual KS incidence rates were 1:4,866 in Malta, 1:8,000 in Sicily and 1:10,970 in Sardinia (Table III).

We investigated the incidence of classical KS and the seroprevalence of KSHV infection in the elderly populations of 3 islands that enclose the central-western part of the Mediterranean Sea. In addition to sharing their location, these 3 islands have a number of similarities and differences in culture, economy and history that may provide insight into the distribution of KSHV and the pathogenesis of classical KS.

Exact KSHV seroprevalence rates, much less exact infection rates, cannot be determined with the current assays. Nonetheless, we believe that our results approximate seroprevalence

**TABLE I**—KSHV SEROPREVALENCE, OVERALL AND BY SEX AND AGE GROUP, IN 3 MEDITERRANEAN ISLANDS

	No. of subjects	Seroprevalence (%) <sup>1</sup>		
		Malta (N = 200)	Sicily (N = 153)	Sardinia (N = 104)
Mean age	457	79 years	76 years	73 years
All subjects		8.5	20.3	25.0
Sex				
Male	244	8.0	20.4	27.5
Female	213	9.0	20.0	22.6
Male				
65–74 years	111	3.1	14.6	29.0
75–84 years	102	9.3	34.4	25.0
>84 years	31	14.3	7.7 <sup>2</sup>	25.0 <sup>4</sup>
Female				
65–74 years	88	3.6	20.0	17.1
75–84 years	92	9.1	26.1	21.4
>84 years	33	9.1	0 <sup>3</sup>	75.0 <sup>4</sup>

<sup>1</sup>By algorithm.<sup>8</sup> Specifically, seropositives have K8.1 OD reactivity > 1.5 or a positive latent IFA if K8.1 OD is at least 0.50. All other samples are seronegative.—<sup>2</sup>N = 13.—<sup>3</sup>N = 7.—<sup>4</sup>N = 4.

reasonably well. Our crude seroprevalence rates are similar to those reported previously for Sicily and Sardinia using IFAs for lytic or latent KSHV antigens.<sup>4,9,10</sup> We have shown recently that one of these assays, the IFA for latent antigens, which was developed in 1995, is complemented by our EIA for antibodies against the KSHV lytic cycle antigens represented in the K8.1 recombinant protein.<sup>8</sup> Specifically, adding the K8.1 EIA increased the sensitivity of the IFA for KS sera from 86% to 90%; more importantly, it increased the specificity of the IFA from 88% to 95%. Whether the K8.1 EIA will have higher reproducibility among laboratories than noted for the IFA<sup>11</sup> remains to be seen.

Previous studies have noted relatively high KSHV seroprevalence rates in Italy, and particularly in Sicily and Sardinia.<sup>4,9,10,12</sup> Ours, however, is the first study of KSHV seroprevalence rates among the elderly. In the 3 islands that we studied, seroprevalence rates varied little from age 65 to 99, and it was quite similar in men and women. By geography, seroprevalence was substantially and statistically significantly lower in Malta than in Sicily or Sardinia.

Geographical differences likely reflect a variety of behavioral, cultural, historical, host genetic and perhaps virological differences in these populations. Sardinia has been relatively isolated, enabling it to maintain a distinct ethnicity and culture. In contrast, Sicily and Malta were more extensively colonized by Phoenicians, Carthaginians, Romans, Byzantines, Arabs and Normans. The histories of Sicily and Malta diverged over the past 5 centuries. Malta was ruled from 1530 to 1798 by the Knights of the Order of St. John and by the British until 1964. Sicily was joined politically with mainland Italy in the 19th century.

Five genotypes of KSHV have been described, of which genotypes A and C are reported to be the most common in Sicily and, in Israel, among Sephardic Jewish populations who had inhabited regions of north Africa and western Mediterranean areas of Europe.<sup>13–15</sup> There are, however, no reported in vivo or in vitro differences in the pathobiology of these KSHV

**TABLE II**—MULTIVARIATE LOGISTIC REGRESSION MODEL OF KSHV SEROPREVALENCE IN 3 MEDITERRANEAN ISLANDS

Variable	OR (95% CI)
Malta	1.00 (referent)
Sicily	3.25 (1.89–5.57)
Sardinia	4.07 (2.25–7.34)
Male	1.00 (referent)
Female	1.20 (0.77–1.86)
Age <76 years	1.00 (referent)
≥ 76 years	1.34 (0.85–2.10)

genotypes. Numerous KSHV variants have been reported from mainland Italy,<sup>16</sup> but no such data have been reported for specimens from Malta or Sardinia.

The elderly men and women in our study had similar KSHV seroprevalence rates but differing incidence rates of classical KS. There appeared to be a gradient in male KS incidence rates (from 2.2 per 10<sup>5</sup> · years in Malta, to 6.2 per 10<sup>5</sup> · years in Sicily, to 8.8 per 10<sup>5</sup> · years in Sardinia) that mirrored the male KSHV seroprevalence rates. Among women, however, classical KS rates were similar in the 3 islands despite significantly lower KSHV seroprevalence rates in Malta. These findings suggest that there are sex-specific co-factors of classical KS and that these co-factors vary geographically. Certain occupational exposures, such as cereal farming and prolonged water exposure,<sup>17,18</sup> could increase the risk of classical KS more for men than for women. Consistent with its lower KS risk, Malta is rocky and dry with very little ground water. There also may be sex-specific differences in genetic susceptibility or resistance to disease, particularly in populations with limited genetic mixing. Sardinia, for example, has high rates for several genetic diseases, such as type I diabetes mellitus,<sup>19</sup> whereas different genetically linked diseases have been reported in Malta.<sup>20</sup>

Our findings may have a more trivial explanation, if KS has a long latency period and if KSHV seroconversions in the elderly are more frequent among women than men. We observed non-significantly higher KSHV seroprevalence rates among women and at older ages. However, a prospective seroincidence study would be needed to test this hypothesis.

Published KS incidence data were available only from the 2 northeastern districts of Sardinia (Sassari and Nuoro, population approximately 700,000) and from the Ragusa Cancer Registry in southeastern Sicily (population approximately 280,000). Thus, these are only fractions of the populations of these 2 large islands. Data from Malta are from a comprehensive survey of physicians and from the National Cancer Registry for all 380,000 inhabitants of this country.<sup>7</sup> Therefore, the data from Malta are likely to be more representative of the whole population than are those from Sardinia and Sicily. However, because the Malta data are based on only 12 classical KS cases, they are susceptible to even small effects of delays in seeking medical attention, inaccuracy in diagnosis and incompleteness in cancer reporting.

Our observation of sex-specific geographical variation in classical KS incidence may be mirrored in the islands of Japan, where incidence rates of adult T-cell leukemia/lymphoma (ATL) vary substantially among male but not female carriers of human T-cell lymphotropic virus type I (HTLV-I). Specifically, among HTLV-I carriers, ATL rates (per 10<sup>5</sup> · year) are 106 in Kyushu, 150 in Shikoku and more than 138 in the Kamigoto

TABLE III—ANNUAL INCIDENCE RATES OF CLASSICAL KS IN 3 MEDITERRANEAN ISLANDS

Years	Malta <sup>2</sup> 1992–1997 (No. cases)	Ragusa, Sicily 1981–1990 (No. cases)	Northeast Sardinia 1977–1991 (No. cases)
Population rate per 100,000 per year <sup>1</sup>			
Male	2.2 (6)	6.2 (26)	8.8 (122)
Female	1.8 (6)	2.5 (12)	2.1 (33)
Male:female ratio	1.2	2.5	4.2
Rate per year among KSHV positives <sup>3</sup>			
Male	28.0/10 <sup>5</sup> = 1:3,574	30.4/10 <sup>5</sup> = 1:3,290	32.2/10 <sup>5</sup> = 1:3,108
Female	20.6/10 <sup>5</sup> = 1:4,866	12.5/10 <sup>5</sup> = 1:8,000	9.1/10 <sup>5</sup> = 1:10,970
Male:female ratio	1.4	2.4	3.5

<sup>1</sup>For subjects ages 50 years and older.—<sup>2</sup>In Malta, there were 12 non-AIDS KS cases over age 50, 6 in males (population 44,671) and 6 in females (population 54,063). Three AIDS-associated KS cases, as well as a 7th classical KS case in a man that occurred at age 44, were not included.—<sup>3</sup>Annual KS incidence:KSHV seroprevalence. Denominators determined by multiplication of population size by seroprevalence rates in Table I.

*Islands among men. The respective rates among women are 61, 58 and 53.<sup>21,22</sup> Thus, ATL rates vary among HTLV-I-infected men, whereas our data suggest that classical KS rates vary among KSHV-infected women. Why disease rates vary by sex is unknown, but these data show that they are not merely the result of differences in virus prevalence. Elucidation of KSHV incidence rates and identification of sex-specific co-factors would enable a deeper understanding of the epidemiology and natural history of KSHV infection and the pathogenesis of KS and perhaps other diseases.*

Francesco VITALE<sup>1</sup>, Dino Vella BRIFFA<sup>2</sup>, Denise WHITBY<sup>3</sup>, Ivana MAIDA<sup>4</sup>, Alicia GROCHOWSKA<sup>5</sup>, Arthur LEVIN<sup>6</sup>, Nino ROMANO<sup>1</sup>, and James J. GOEDERT<sup>7\*</sup>

<sup>1</sup>Department of Hygiene and Microbiology “Giuseppe D’Alessandro,” University of Palermo, Sicily, Italy

<sup>2</sup>Department of Dermatology, Sir Paul Boffa Hospital, Malta

<sup>3</sup>Viral Epidemiology Laboratory, SAIC-National Cancer Institute, Frederick, MD, USA

<sup>4</sup>Istituto di Igiene, Università degli studi di Sassari, Sassari, Sardinia, Italy

<sup>5</sup>Department of Hematology, St. Luke’s Hospital, Malta

<sup>6</sup>Department of Virology, St. Bartholomew’s Hospital Medical School, London, United Kingdom

<sup>7</sup>Viral Epidemiology Branch, National Cancer Institute, Rockville MD, USA

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\*Correspondence to 6120 Executive Blvd., Room 8012, Rockville, MD 20852, USA. FAX: 301-402-0817.  
E-mail: GoedertJ@mail.nih.gov

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